Environmental Science & Technology

Article

pubs.acs.org/est

Effects of Increasing Nitrogen and Phosphorus Concentrations on Phytoplankton Community Growth and Toxicity During *Planktothrix* Blooms in Sandusky Bay, Lake Erie

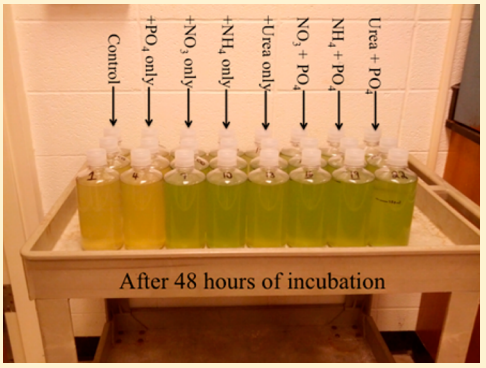
Timothy W. Davis,*[,†,§] George S. Bullerjahn,[‡] Taylor Tuttle,[‡] Robert Michael McKay,[‡] and Susan B. Watson[†]

[†]Canada Centre for Inland Waters, Environment Canada, Burlington, Ontario L7S 1A1, Canada

[‡]Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, United States

Supporting Information

ABSTRACT: Sandusky Bay experiences annual toxic cyanobacterial blooms dominated by *Planktothrix agardhii/suspensa*. To further understand the environmental drivers of these events, we evaluated changes in the growth response and toxicity of the *Planktothrix*-dominated blooms to nutrient amendments with orthophosphate (PO_4) and inorganic and organic forms of dissolved nitrogen (N; ammonium (NH_4), nitrate (NO_3) and urea) over the bloom season (June − October). We complemented these with a metagenomic analysis of the planktonic microbial community. Our results showed that bloom growth and microcystin (MC) concentrations responded more frequently to additions of dissolved N than PO_4, and that the dual addition of NH_4 + PO_4 and Urea + PO_4 yielded the highest MC concentrations in 54% of experiments. Metagenomic analysis confirmed that *P. agardhii/suspensa* was the primary MC producer. The phylogenetic distribution of *nifH* revealed that both heterocystous cyanobacteria and heterotrophic proteobacteria had the genetic potential for N_2 fixation in Sandusky Bay. These results suggest that as best management practices are developed for P reductions in Sandusky Bay, managers must be aware of the negative implications of not managing N loading into this system as N may significantly impact cyanobacterial bloom size and toxicity.



INTRODUCTION

Cyanobacterial harmful algal blooms (CHABs) threaten the Laurentian (North American) Great Lakes, a major freshwater resource containing roughly 18% of Earth's available surface freshwater.[1] Over the past several decades these ecosystems have been subject to many anthropogenic pressures such as nutrient overenrichment (eutrophication), contaminant inputs, structural engineering (impoundments, shoreline development and dredging), wetland loss, and invasive species (e.g., dreissenid mussels and round gobies). Lake Erie is the most socio-economically important of the Great Lakes,[1−3] serving the recreational, commercial, and drinking water needs of over 11.5 million people.[3] Lake Erie is also is the smallest and shallowest Great Lake, making it the most susceptible to increased nutrient loading. Lake Erie has been impacted by poor water quality, including CHABs, as far back as the 1960s.[4−6] However, with the introduction of phosphorus reduction programs in 1972 as part of the Great Lakes Water Quality Agreement,[7] the water quality (e.g., reduction in cyanobacterial biomass) in Lake Erie improved significantly throughout the late 1970s and 1980s.[6,8] However, since the mid-1990s, Lake Erie and its watershed have been increasingly affected by toxic CHABs, largely fuelled by agricultural nonpoint nutrient sources.[9−11] To date there has been no consensus on the key factors driving HAB occurrence, extent and timing, even in well-studied systems such as Lake Erie.[12]

Traditionally, it has been thought that phosphorus (P) was the primary limiting nutrient in freshwater systems,[13,14] including Lake Erie.[9,15] However, the importance of nitrogen (N) in the control of eutrophication and CHAB events has been gaining attention.[16−18] Previous studies on Lake Erie blooms have investigated bottom-up controls such as nutrient availability and light,[19−21] physical factors like wind strength[22] and top-down controls including pelagic[23] and benthic grazing.[24,25] Furthermore, differences and dynamics among the genetic strains of cyanobacteria within Lake Erie blooms have been investigated through field and laboratory experiments.[11,26,27]

Planktothrix, a globally distributed genus,[28] is less common than *Microcystis* in the offshore Lake Erie blooms but can predominate in bays and tributaries, notably Sandusky Bay[29] and the Maumee River.[30] *Anabaena* (currently called *Dolichospermum*[31]) spp. blooms also occur in the western and

Received: February 12, 2015
Revised: May 18, 2015
Accepted: May 20, 2015
Published: May 20, 2015

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

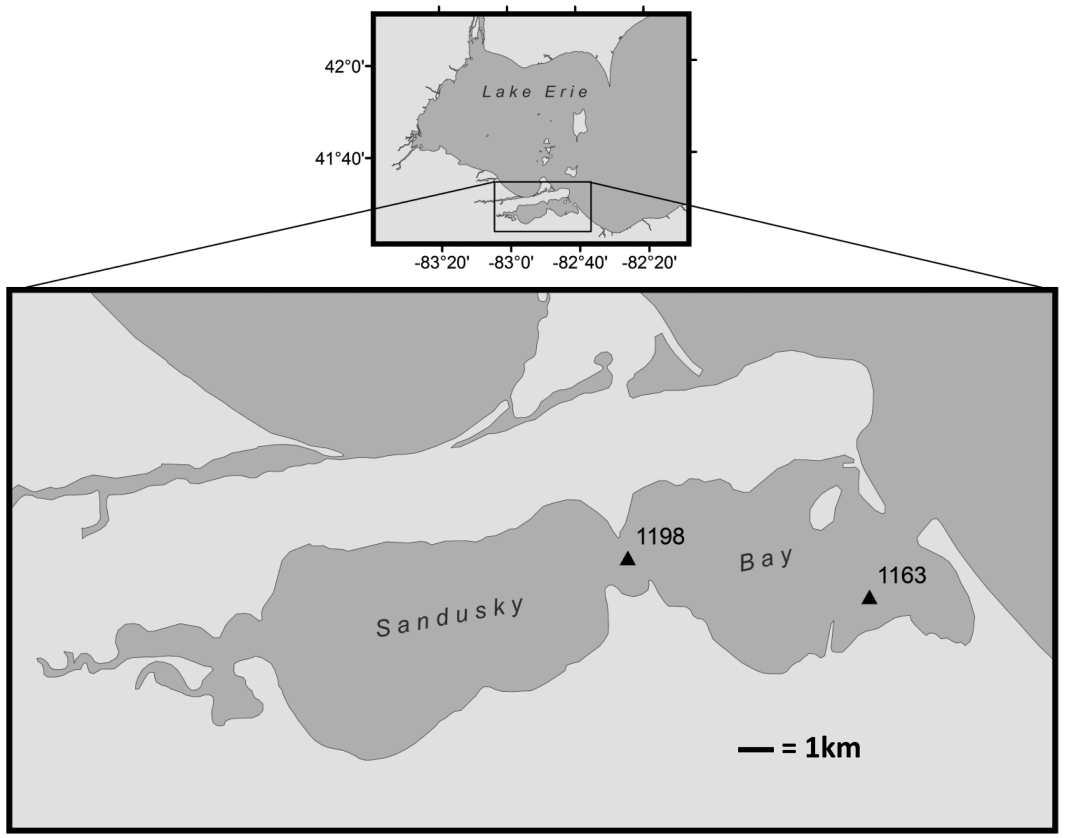


Figure 1. Map of Sandusky Bay, Lake Erie with the two experimental sites, EC 1163 and EC 1198 (black triangles).

central basins of Lake Erie[21,23,32] but to date, the ecology and toxicity of these events have been largely overlooked.

summer monthsOnly a few studies have investigated the phytoplankton population in Sandusky Bay,[29] bloom phylogenetics[33] and the growth response of the *Planktothrix* community to N or P additions.[34] Rinta-Kanto and Wilhelm[33] identified *Planktothrix* spp. as the primary MC-producers in Sandusky Bay. Chaffin and Bridgeman[34] have investigated the short-term growth response of the total phytoplankton community to N and P amendments, but did not examine toxins and their study was limited to a single experiment (July) in Sandusky Bay. Furthermore, even though it has been demonstrated that DIN in Sandusky Bay decreases rapidly during the summer months, the non-diazotrophic cyanobacterium, *Planktothrix*, dominates throughout the growing season. However, little is known about the Sandusky Bay N-fixing community, except that the diazotrophic cyanobacterium, *Cylindrospermopsis raciborskii*, can be present at measureable densities.[23,29] A better understanding of the composition of the N-fixing community may provide significant insight into the ecological reasons why a non-diazotrophic cyanobacterium can dominate a chronically N-limited system. Therefore, our study aimed to evaluate changes in the growth response of the *Planktothrix*-dominated blooms to increases in inorganic phosphorus and inorganic and organic forms of nitrogen over the growing season (June−October) while complementing these results with measurements of microcystin concentrations and metagenomic data on the planktonic microbial community.

MATERIALS AND METHODS

Study Sites. Sandusky Bay is a shallow (mean depth: 2.6 m[29]), well-mixed drowned river mouth at the southeast corner of the western basin of Lake Erie (Figure 1). Sandusky Bay has an hourglass shape and is divided into the outer bay (eastern half) and the inner bay (western half; Figure 1). During May through October 2013, short-term nutrient amendment experiments were conducted on samples from the outer bay at two Environment Canada sites (EC 1198; EC 1163, Figure 1). Samples were collected from a small boat (EC 1198) or from the CCGS *Limnos* (both sites); EC 1163 was accessible only from the CCGS *Limnos*, and due to ship scheduling no sampling or experiments occurred between early June and mid-July (Supporting Information Tables 1 and 2, respectively).

Sample Collection. Physicochemical data were measured at each site at approximately the same time each sampling trip using a calibrated water quality probe (YSI 6600, Yellow Springs, OH), measuring surface water temperature, dissolved oxygen concentration, pH, and conductivity. Water samples (60 L) were collected using a rosette from a depth of 1 m and either processed immediately when onboard CCGS *Limnos* or kept at room temperature until returned to Bowling Green State University (BGSU) for processing within 2 h. From each site, subsamples were processed for total chlorophyll *a* (chl *a*; 47 mm GF/F), particulate microcystins (MCs; 1.2 μm polycarbonate membrane) and cell-bound DNA analysis (0.22 μm Sterivex filter cartridge; Millipore Corp., Billerica, MA). The filter cartridges were immediately frozen with chl *a* and MC samples stored at −20 °C and the Sterivex filter cartridges stored at −80 °C until analysis. Samples for phytoplankton community composition and biomass determination were preserved with Lugol's iodine solution (1% v/v. final conc.). Rainfall data for the area surrounding Sandusky Bay were collected from the U.S. Climate Data Web site (http://www.

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

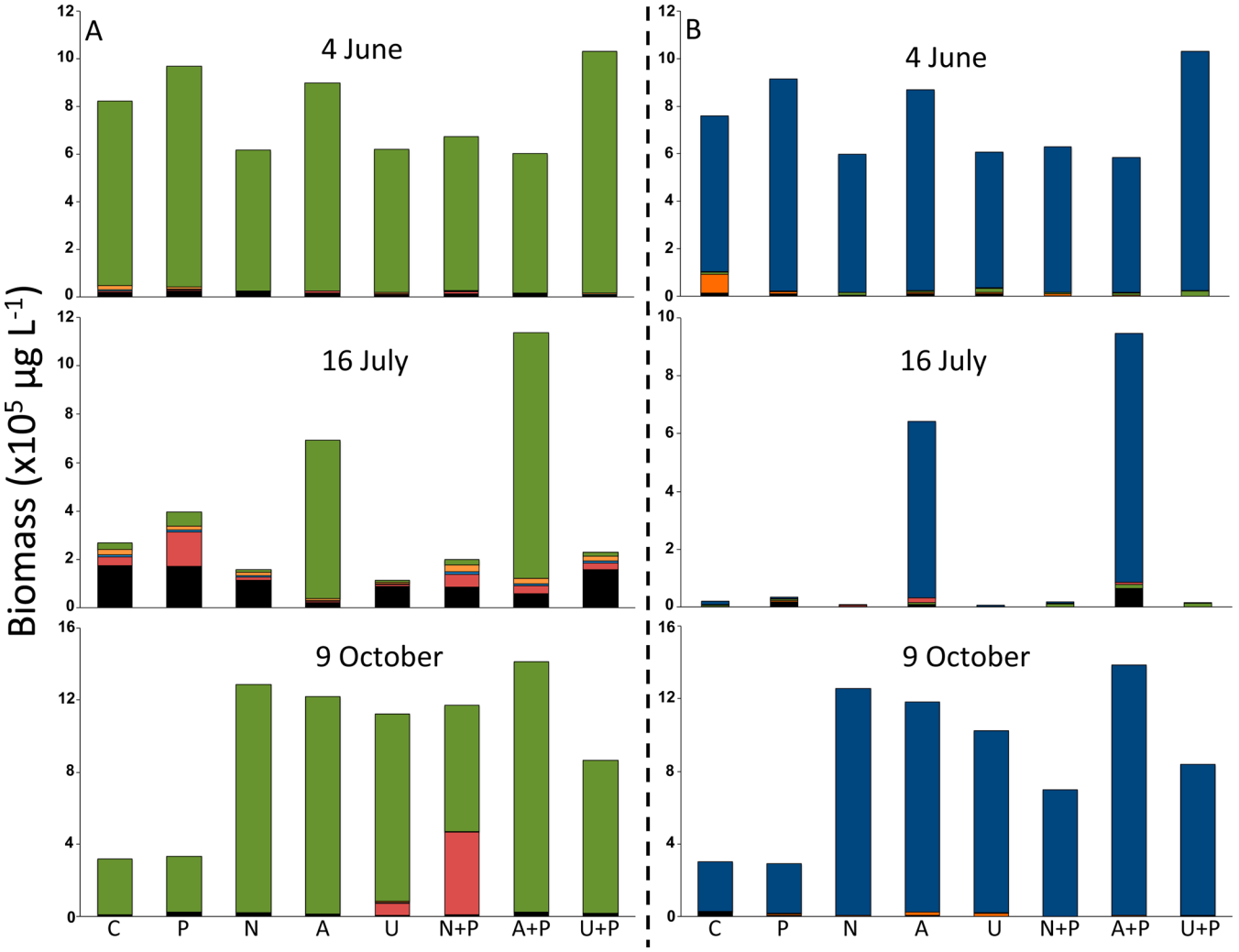


Figure 2. Representative seasonal phytoplankton community biomass responses from experiments conducted at site 1163. (A) Total phytoplankton biomass, Green = Cyanobacteria, Orange = Cryptophyta, Light Blue = Chrysophyta, Red = Chlorophyta, Black = Bacillariophyta. (B) Potentially-toxic cyanobacteria, Dark Blue = *Planktothrix agardhii/suspensa*, Red = *Limnothrix* spp., Green = *Cuspidothrix issatschenkoi*, Orange = *Aphanizomenon* spp., Black = *Anabaena/Dolichospermum* spp. For the *x*-axis treatment abbreviations: C = control, P = orthophosphate, N = Nitrate, A = Ammonium and U = Urea.

usclimatedata.com/). Weekly average rainfall was calculated from May through October 2013.

Duplicate water samples for the analyses of dissolved nutrients (nitrate/nitrite [NO_3^- + NO_2^-], ammonia [NH_3] and soluble reactive phosphorus [SRP]) were collected by filtering lake water through a 0.45 μm polycarbonate filter into triple-rinsed 20 mL plastic bottles and stored at −20 °C until analysis. Water samples for total phosphorus [TP] analysis were collected by filling a triple-rinsed 20 mL plastic vial with whole lake water followed by storage at −20 °C. All samples were analyzed at the National Center for Water Quality Research, Heidelberg University, Tiffin, OH using standard techniques.[35]

Nutrient Amendment Experiments. Short-term nutrient enrichment experiments were conducted to assess the impact of increased organic and inorganic N or PO_4 concentrations on the *Planktothrix agardhii/suspensa* populations in Sandusky Bay. During the summer of 2013, a total of 13 experiments were conducted on samples from sites EC 1163 and EC 1198. To commence experiments, sets of triplicate, 1 L clear polycarbonate bottles (n = 24) were filled with surface water from each experimental site and were either left unamended to serve as a control, amended with orthophosphate (KH_2PO_4; referred to as PO_4 from hereon) various forms of N: nitrate (combination of $Ca(NO_3)_2$ plus KNO_3), ammonium (NH_4Cl), or urea, or a combination of each individual N form and PO_4. All N treatments were amended to a final concentration of 178.9 μM and all P treatments were spiked to a final concentration of 1 μM. In order to facilitate comparisons between these field experiments and planned laboratory experiments, the N concentration of 178.9 μM was chosen because it represented 10% of the total N concentration in the growth media used in our laboratory. Experiments were conducted in two locations (See Supporting Information Table 2). Those carried out onboard the CCGS *Limnos* were incubated in a flow-through deck incubator under ambient water and light conditions. Neutral density screening was used to achieve light levels similar to in situ conditions. The experiments conducted at BGSU were incubated in a stand-alone incubator using temperatures and light levels (50 μmol m^{-2} s^{-1}) similar to measured field conditions on each sampling date and seasonally appropriate photoperiods (14:10 h: June, July, and August; 12:12 h: September and October). Incubator

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

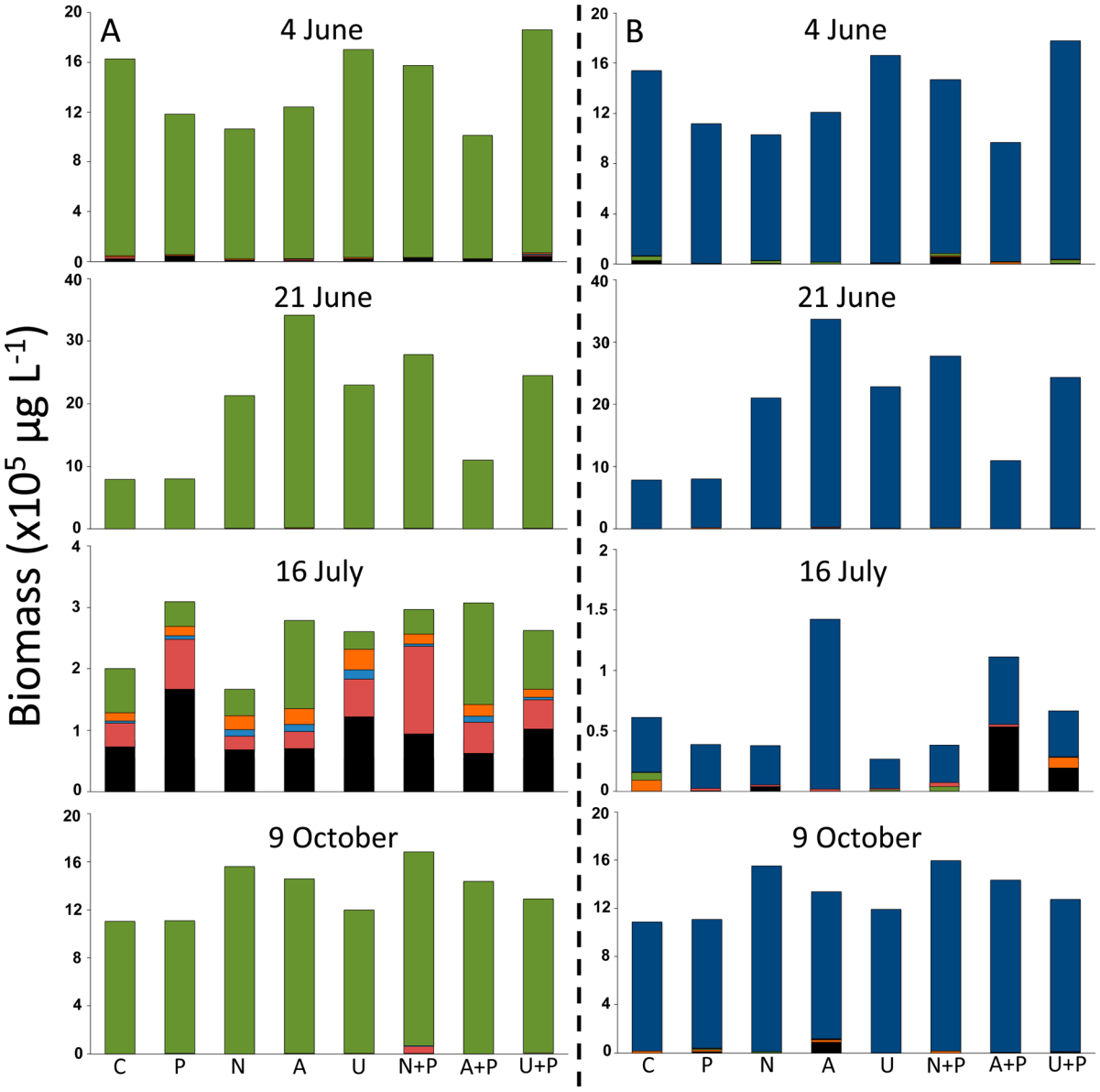


Figure 3. Representative seasonal phytoplankton community biomass responses from experiments conducted at site 1198. (A) Total phytoplankton biomass, Green = Cyanobacteria, Orange = Cryptophyta, Light Blue = Chrysophyta, Red = Chlorophyta, Black = Bacillariophyta. (B) Potentially-toxic cyanobacteria, Dark Blue = *Planktothrix agardhii/suspensa*, Red = *Limnothrix* spp., Green = *Cuspidothrix issatschenkoi*, Orange = *Aphanizomenon* spp., Black = *Anabaena/Dolichospermum* spp. For the *x*-axis treatment abbreviations: C = control, P = orthophosphate, N = Nitrate, A = Ammonium and U = Urea.

temperatures during all experiments were measured every 5 min with in situ loggers (Onset Computer Corporation, Bourne, MA) and indicated that incubation temperatures and light levels remained within the same range (i.e., light: 50−100 μmol m^{-2} s^{-1}; temperature ambient ±1 ° C) as those found at each site. All bottles were gently inverted every 4−6 h. After 48 h, each bottle was sampled as described above to quantify chl *a*, phytoplankton community composition and biomass and particulate concentrations of MCs.

The effects of P and inorganic and organic N on the chl *a* concentration and MCs concentration were analyzed with one-way ANOVAs. Posthoc comparisons of significant impacts were elucidated with Tukey's multiple comparison tests. For all results, the standard variance presented is ± one standard error (SE). Since only one replicate per treatment was enumerated for phytoplankton community composition and biomass, statistical tests could not be performed.

Extraction and Analysis of Microcystins. Particulate MCs were extracted from samples using a combination of physical and chemical lysis techniques. All samples were resuspended in 1 mL molecular grade water (pH 7; Sigma-Aldrich, St. Louis, MO) and subjected to three freeze/thaw cycles before the addition of the QuikLyse reagents (Abraxis LLC; Warminster, PA) as per the manufacturer's instructions. The samples were then centrifuged for 5 min at 2 × 10^3*g* to pellet cellular debris. The concentrations of microcystins (reported as microcystin-LR equivalents) were measured using an enhanced sensitivity microcystin enzyme-linked immunosorbent assay (Abraxis LLC) following the methodologies of Fischer et al.[36] This assay is congener-independent as

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

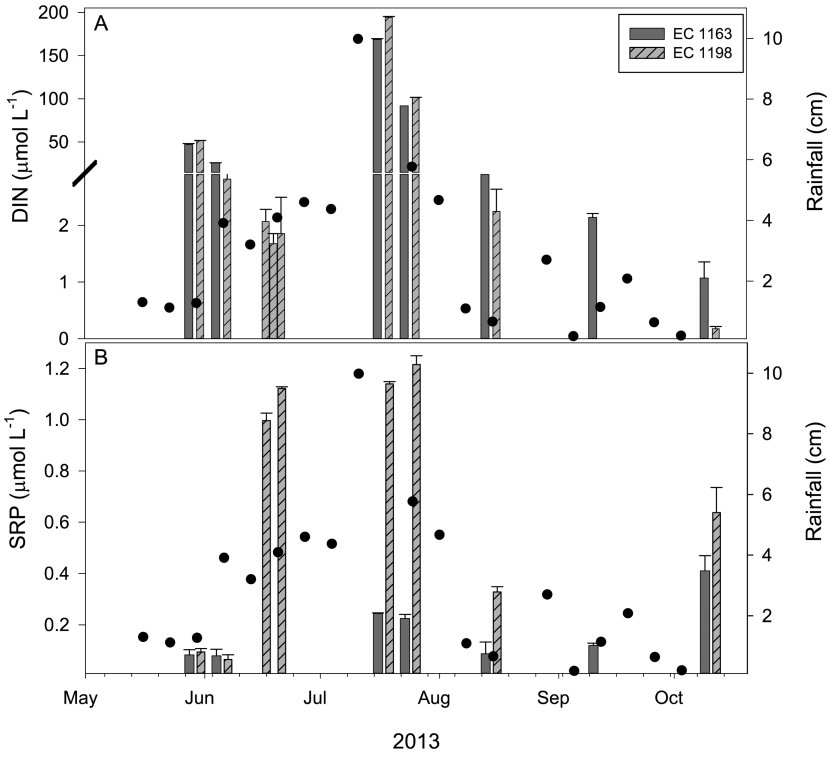


Figure 4. Nutrient and rainfall data for Sandusky Bay during the bloom season of 2013. (A) Solid and striped columns represent dissolved inorganic nitrogen (DIN) concentrations at sites EC 1163 and EC 1198, respectively (note the axis break). Rainfall data is represented by the black circles. (B) Solid and striped columns represent soluble reactive phosphorus (SRP) concentrations at sites EC 1163 and EC 1198, respectively. Error bars for the DIN and SRP data represent 1 SE of duplicate samples.

it detects the ADDA moiety, which is found in almost all MCs. These analyses yielded a detection limit of 0.04 μg L^{-1}.

Phytoplankton Community Composition and Biomass. After evaluating the chlorophyll *a* data, one replicate per treatment in each of the seven representative seasonal experiments (Figures 2 and 3) was chosen for enumeration to support the pigment-based results. Further, since the initial chlorophyll *a* concentrations and control bottles after 48 h were statistically similar in all but one experiment we only enumerated the control from each experiment and not the initial. Aliquots of the preserved samples from were allowed to settle overnight in sedimentation chambers following the procedure of Lund et al.[37] Algal units were counted from randomly selected transects on a Zeiss Axiovert 40 CFL inverted microscope. Counting units were individual cells, filaments or colonies depending on the organization of the algae. A minimum of 400 units were counted for each sample. The bulk of the counts are made at 600× magnification. The large and rare organisms (such as *Ceratium*) were scanned for at 350× magnification. Fresh weight biomass was calculated from recorded abundance and specific biovolume estimates, based on geometric solids,[38] assuming unit specific gravity (1.0 g = 1.0 cm^3). The biovolume (mm^3 m^{-3} fresh weight) of each species was estimated from the average dimensions of 10−15 individuals. The biovolumes of colonial taxa were based on the number of individuals in a colony. All calculations for cell concentration and biomass were performed with Hamilton's[39] computer program.

Sandusky Bay Metagenome. Microbial biomass from 200 mL surface water collected on 17 June 2013 at EC 1198 was concentrated on Sterivex cartridge filters (0.22 μm) and immediately frozen in liquid nitrogen. DNA was extracted from the Sterivex cartridges using the PowerWater Sterivex DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) following manufacturer's instructions. The metagenome was obtained by Illumina sequencing on a HiSeq platform at the DOE-Joint Genome Institute (Walnut Creek, CA). The annotated metagenome data set comprised 7.3 × 10^9 total bases and 3.8 × 10^7 total sequences. Cyanobacterial and bacterial metagenome scaffolds containing genes of interest (*ntcA*, *nifHDK*, *mcyA*) were identified via BLAST,[40] followed by alignment by CLUSTAL W and phylogenetic analysis in MEGA.[41] Trees were constructed by neighbor-joining with 1000 bootstrap replicates.

Accession Numbers. Sequences were deposited into the JGI IMG database (IMG Taxon OID 3300003684).

RESULTS

Water Quality and Rainfall Data. The Sandusky Bay bloom persisted from May through October, 2013. At both sites, surface water temperatures ranged from 16.6−27.0 °C, dissolved oxygen concentrations were generally supersaturating (mean: 10.7 ± 1.1 mg L^{-1}) and the pH and conductivity were consistent throughout the sampling period (mean: 8.4 ± 0.1 and 401 ± 20 μS cm^{-1}, respectively). Chlorophyll *a* concentrations of all samples at both stations ranged from 18.4 ± 0.2 μg L^{-1} to 66.4 ± 7.7 μg L^{-1} (Supporting Information Table 1). Cyanobacteria dominated the phytoplankton community biomass (95−99%) at all times except during mid-July when cyanobacteria only comprised 11% and 32% of the total phytoplankton biomass at stations 1163 and 1198, respectively (Figures 2 and 3). *Planktothrix agardii/suspensa* dominated the cyanobacterial community comprising >73% of the biomass on all but one date (July 16) at site 1163

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

(Figures 2 and 3). In spring and early summer, concentrations of MCs frequently exceeded the State of Ohio's Public Health Advisory guideline level of 6 $\mu g\ L^{-1}$ with values falling below this limit in midsummer (Supporting Information Table 1). May and June samples ranged from 5.2 ± 1 $\mu g\ L^{-1}$ to 11.7 ± 2.6 $\mu g\ L^{-1}$, peaking on June 19 at EC 1198 (Supporting Information Table 1). By contrast, July − October concentrations of MCs ranged from a midsummer low of 0.4 ± 0.1 $\mu g\ L^{-1}$ on July 16 at EC 1198 and increased to 5.3 ± 0.5 $\mu g\ L^{-1}$ on October 9 (Supporting Informaiton Table 1).

Profiles of nutrient concentrations (SRP and DIN) measured at the time of each experiment indicated that whereas SRP remained well above DL and showed only moderate changes with time (EC 1163:0.08 ± 0.03−0.41 ± 0.06 μM; EC 1198: 0.06 ± 0.02−1.21 ± 0.03 μM, Figure 4B), DIN was highly variable (Figure 4A). DIN declined at both stations in late August − October, and at EC 1198 in late May and early June. However, DIN concentrations peaked in late June and early July at values almost 100 fold higher, following a series of midsummer rain events and increased runoff (Figure 4; www.usclimatedata.com/climate/sandusky/ohio/united-states/usoh0855/2013/8).

Nutrient Amendment Experiments. In total, seven experiments were conducted at EC 1163 and six at EC 1198. A representative experimental date was chosen from early spring, early summer midsummer and early fall for a visual comparison of seasonal responses both within and between stations. 1163 had no early summer experiment (Figures 2, 3, 5 and 6). Both stations showed similar overall responses in algal growth, (as inferred by increasing chl *a* concentrations) and concentrations of MCs as both were primarily stimulated by N in experiments conducted during the summer of 2013 (Figures 5 and 6; Supporting Information Table 2). However, each station yielded different responses to nutrient additions at different points throughout the summer.

During early summer (late May - early June), concentrations of chl *a* and MCs did not exhibit significantly positive responses from the control in any treatment ($p > 0.05$; Figures 5 and 6; Supporting Information Table 2). Phytoplankton biomass response was consistent with the overall chl *a* response and no shift in community composition was observed (Figures 2 and 3). No experiments were conducted at EC 1163 during June, but all experiments conducted throughout this month at 1198 indicated that by mid-June, algal growth was strongly N-limited, with additions of any form of N yielding chl *a* concentrations that were nearly double the control treatments (Figure 6; Supporting Informaiton Table 2). MC concentrations responded to the addition of N in all three experiments, suggesting that bloom toxicity was also strongly N-limited ($p < 0.05$; Figure 6; Supporting Information Table 2). Further, phytoplankton biomass revealed that the chl *a* response was due to increases in cyanobacterial community biomass dominated by *Planktothrix agardhii/suspensa* (Figure 3). For all forms of N, there was no significant interactive effect with PO_4 in either chl *a* or MC concentrations (Figure 6; Supporting Information Table 2).

In July (where both sites were tested), the phytoplankton community was codominated by diatoms, green algae and cyanobacteria (Figures 2 and 3) at 1163 and 1198 with cyanobacteria only comprising 11 and 32% of the community biomass, respectively. Overall inferred phytoplankton growth at both sites was influenced by both N and P treatments. At site 1198 overall growth was minimal and the increase in chl *a*

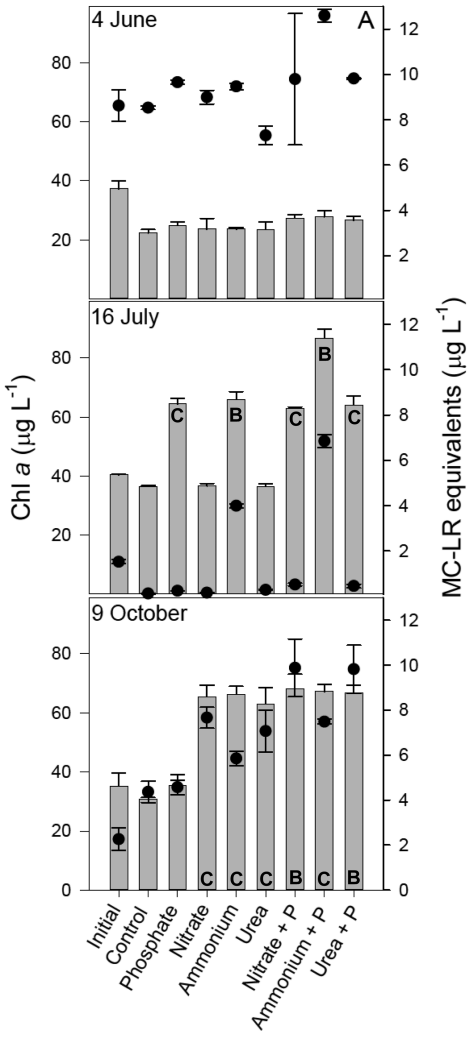


Figure 5. Representative seasonal community responses during nutrient amendment experiments at site EC 1163. Total chlorophyll *a* is represented by the gray bars and particulate microcystins are represented by the black circles. Error bars represent ±1 SE of triplicate bottles. B = both concentrations of chlorophyll *a* and particulate microcystins significantly increased above the control. C = only concentrations of chlorophyll *a* significantly increased above the control.

concentration (Figure 6) was due to a combination of diatom, green algae and cyanobacterial growth (Figure 3). However, the increase in MCs, while also minimal, were likely due to changes in *P. agardhii/suspensa* biomass (Figure 3). Similarly, chl *a* response at site 1163 were due to a combination of diatom, green algae and cyanobacterial growth (Figure 2). The NH_4 and NH_4 + P treatments yielded a community shift toward cyanobacterial dominance (Figure 2) and significant increases in concentrations of MCs (Figure 5).

By late summer-early fall, the phytoplankton communities at both sites were once again dominated by cyanobacteria, with *P. agardhii/suspensa* comprising most of that biomass (Figures 2 and 3). Inferred phytoplankton growth at both stations was again constrained by N as chl *a* concentrations significantly increased in all individual N treatments and was not further stimulated by any single or combined P treatments (Figures 5 and 6; Supporting Information Table 2). However, concentrations of MCs did not respond to individual forms of N as

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

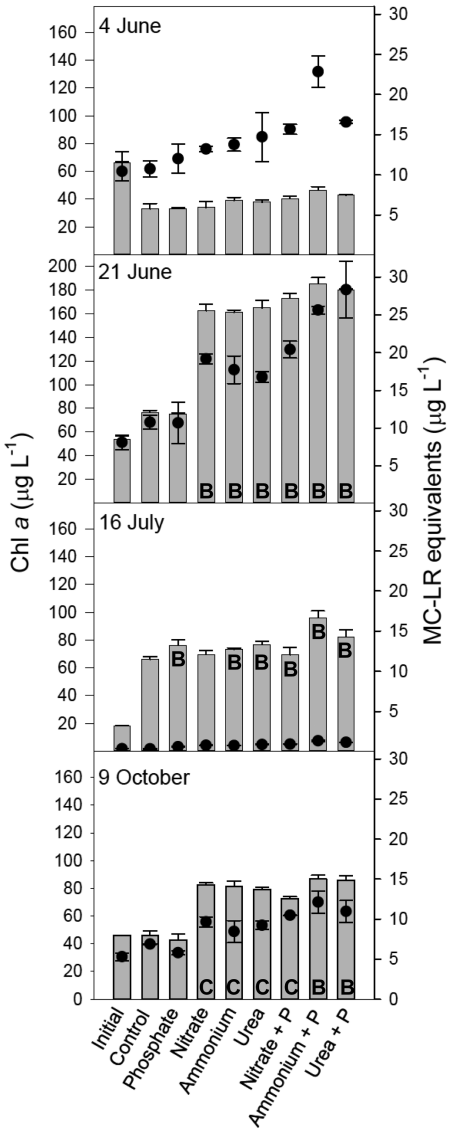


Figure 6. Representative seasonal community responses during nutrient amendment experiments at site EC 1198. Total chlorophyll *a* is represented by the gray bars and particulate microcystins are represented by the black circles. Error bars represent ±1 SE of triplicate bottles. B = both concentrations of chlorophyll *a* and particulate microcystins significantly increased above the control. C = only concentrations of chlorophyll *a* significantly increased above the control.

they did in June as the production of MCs tended to be dual limited by N and P.

Cyanobacterial Diversity in Sandusky Bay. Penno et al.[42] demonstrated the use of the cyanobacterial *ntcA* nitrogen regulation gene to assess phylogenetic relationships among members of the phylum. Based on this criterion, our analysis of the 2013 Sandusky Bay bloom metagenome indicated a very low diversity of the cyanobacterial community (Supporting Information Figure 1). Three *ntcA* genotypes were detected, dominated by *Planktothrix agardhii/suspensa* (2171 reads). Present at low copy number were a single Nostocales genotype (22 reads) and an Oscillatoriales *ntcA* (8 reads). Regarding toxigenicity of the bloom, synthesis of MCs was attributed exclusively to *P. agardhii/suspensa* based on analysis of *mcyA* sequences (data not shown).

Nitrogen Fixing Community in Sandusky Bay. As might be expected, our analysis showed *nifH* clusters associated with several major cyanobacterial groups, including *Nostoc* and *Dolichospermum* lineages (Supporting Information Figure 1). However, a large number of *nifH* sequences were from Proteobacteria (Supporting Information Figure 2), and in fact, phylogenetic assignment indicated that these heterotrophs accounted for the majority (58%) of the *nifH* sequences, with Cyanobacteria contributing 32% of the sequences (Supporting Information Figure 2). The other 10% of the reads were from a combination of other heterotrophic bacteria (Supporting Information Figure 2).

DISCUSSION

Seasonal Variability in Sandusky Bay Bloom Biomass and Toxicity. This study was the first to investigate the seasonal response of chl *a*, phytoplankton biomass and MCs to increasing N and P concentration during cyanobacterial blooms dominated by *Planktothrix* in the Great Lakes. Phytoplankton biomass was dominated by *P. agardhii/suspensa* for nearly the entire summer except during mid to late July when diatoms and green algae co-dominated and concentrations of MCs were minimal. Levels of MCs were far more variable than chl *a*, with the bloom more toxic early in the season than midsummer, and a modest recovery in toxicity in October. It is likely that seasonal shifts of phytoplankton genera as well as changes between toxic and nontoxic *Planktothrix* genotypes contributed to changes in toxicity through the summer into fall. Similar studies on toxic and nontoxic *Microcystis* populations throughout the northeast U.S.[16,43,44] and *Microcystis* and *Planktothrix* populations elsewhere[45−47] have shown that differences between toxic and nontoxic strains vary temporally and spatially. Furthermore, nutrient speciation and ratios have been shown to influence the proportion of toxic to nontoxic cells.[16]

Nutrient Effects on Biomass and Toxicity. Concentrations of chl *a* were stimulated by the addition of any form of N in over half of all experiments. All of the dates when neither N nor P yielded a significant effect in any treatment occurred in early summer when water temperatures were between 16 and 21 °C, well below optimal growth for *P. agardhii* (25 °C[48]). Therefore, while *P. agardhii/suspensa* dominated the community biomass, the growth response may have been too slow to see any changes in 48 h. P additions only increased chl *a* concentrations in midsummer (July 16 and 23) when increased N availability followed a series of large rain events that likely delivered N as agricultural runoff to Sandusky Bay. Interestingly, we did not measure a parallel increase in SRP concentrations following the rain events but this may have been due to the P entering Sandusky Bay as particulate P and hence would not be detected with our methods. The dual additions of N and P resulted in significantly enhanced growth in 77% of experiments but these responses were largely due to N additions, as additive increases in chl *a* concentrations were only observed in 15−23% of the experiments depending on which form of N was used. Previous studies have also shown that the cyanobacterial growth in Sandusky Bay can be N-limited during the summer months[34] but that conclusion was based on a study with limited temporal resolution. Our results broaden that finding and are similar to other mechanistic studies that investigated the seasonal response of non-diazotrophic cyanobacteria (e.g., *Microcystis* and *Planktothrix*)

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

and toxicity to N and P additions in a naturally P-rich temperate lake between June and August.[49,50]

Similarly, individual N additions yielded significantly higher concentrations of MCs more frequently than increases in PO_4. However, the dual addition of N and PO_4 yielded significantly higher concentrations of MCs with the treatment NH_4^+ and P yielding significantly different results than the addition of either nutrient separately in three experiments. While the overall response of MCs to nutrient additions was more variable than shown for chl *a*, the results of this study were similar to those of Donald et al.[49] that reported increases in MCs more frequently by the addition of N than by P.

Planktothrix spp. often dominate the algal community in turbid, eutrophic, shallow waters,[30,51,52] as is the case in Sandusky Bay, Lake Erie. Sandusky Bay comprises the mouth of the Sandusky River at the site it enters Lake Erie. Delivery of sediment from the river contributes to poor light penetration (Secchi depth <30 cm) and high particulate nutrient loadings. While the interactive effects of light intensity and nutrient concentrations were not addressed in this study, light limitation may play an important role in limiting overall growth of the CHABs in Sandusky Bay. In order to address this, larger mesocosm studies must be conducted. However, with respect to nutrients, Sandusky Bay exhibits characteristics distinct from the open waters of Lake Erie. Indeed, Conroy and colleagues[29] showed that molar N:P ratios in Bay waters are far lower than in offshore sites in the lake, trending below the Redfield ratio of 16 during late spring into summer.

Although N-fixing *Anabaena*, *Aphanizomenon*, *Cuspidothix*, and *Cylindrospermopsis* are often present in the Bay,[23,29] the nondiazotrophic *P. agardhii* dominates the community. Similarly, other studies report *Planktothrix* spp. as dominant in N-limited, P-rich waterbodies including Grand Lake St. Marys (Ohio)[11] and Wascana Lake (Saskatchewan, Canada;[49,50]). In Transquaking River, MD[16] and Lake Taihu, China[53] where there is chronic N limitation during the summer months, the algal community is similarly dominated by a nondiazotrophic, toxic cyanobacterium, *Microcystis*. Importantly, our work has bearing on two traditional paradigms, both of which are increasingly challenged by new data. First, it has been long asserted that low N:P ratios may favor diazotrophic cyanobacteria[54] but this is now debated,[51,55,56] and questions remain as to the capacity of *Planktothrix* and other nondiazotrophic cyanobacteria to scavenge N, and the sources of new N into the system that these bloom-forming cyanobacteria can exploit. Second, our observations are consistent with a growing body of literature showing that in many freshwater systems, including Lake Erie, seasonal N limitation may constrain cyanobacterial blooms,[11,57] complicating the traditional view that P limits phytoplankton growth in lakes (Schindler[13,14]).

Metagenome Analysis of Sandusky Bay Cyanobacterial Blooms. As expected, *P. agardhii/suspensa* dominated the cyanobacterial *ntcA* reads, with two other taxa represented. One of these is a member of the Nostocales and is thus likely a diazotroph. Examining *mcyA* reads as a proxy for cyanobacterial strains genetically capable of producing MCs revealed that all the toxigenic forms were *P. agardhii/suspensa*, but the lower number of *mcyA* reads (300) compared to the *ntcA* (2171) suggests that the majority of the *Planktothrix* strains present are nontoxic.

The metagenome also provided a snapshot of the endemic N fixers that may support the N-limited *Planktothrix* bloom. Examining the phylogenetic distribution of *nifH* reads revealed that both heterocystous Cyanobacteria and heterotrophic Proteobacteria exhibited the genetic potential to fix nitrogen. Since Sandusky Bay is a eutrophic system with abundant organic carbon,[58] relationships between heterotrophic bacteria capable of fixing nitrogen (free-living and epiphytic) and nondiazotrophic cyanobacteria cannot be overlooked[59,60] which may lead to more effective "phycosphere"[61] N and P recycling. These relationships have never been explored for *Planktothrix* spp. Furthermore, benthic N fixation may also play a role in the N cycle of Sandusky Bay and other similar systems, but to date no study has investigated this.

Implications for Nutrient Management Strategies. Microcystins are N-rich compounds (on average 10 N atoms per molecule) and previous studies have found that MCs can represent up to 2% of the cellular dry weight of *Microcystis*.[62] Thus, it is likely that the synthesis of MCs will create additional N requirements.[63] Indeed, Van de Waal et al.[64,65] found that the synthesis of N-rich secondary metabolites increased with N enrichment. Other laboratory studies have demonstrated clear relationships between DIN supply and the productions of MCs in *Microcystis*.[66] Our results also support these conclusions and further support previous findings that N supply and to some extent, N speciation, play important roles in the toxicity of *Planktothrix* blooms in P-rich systems.[16,49] Current estimates of the impact of anthropogenic activities on N supply to the biosphere predict an increase of N by 2-fold by 2050.[67] Positive correlations exist between N supply and MC-producing cyanobacteria in P-rich lakes,[68,69] therefore regulating N supply in these systems is critical for reducing bloom toxicity. The results of this study combined with the aforementioned studies, and other laboratory,[64,70] microcosm,[16,34] mesocosm[71−73] and whole lake[74,75] studies strengthen the argument that in certain systems, N management is equally as important as P management for the reduction of bloom biomass and toxicity. Furthermore, Sandusky Bay must have strong N-sinks such as assimilatory N-reduction and dissimilatory sediment N-reduction given the high organic carbon inputs,[58] but to our knowledge no study has investigated these processes in Sandusky Bay and management efforts would certainly need to consider these processes.

Our conclusions do not suggest that P reductions are not necessary; on the contrary, this system is N-limited because P-loading to Sandusky Bay is high and therefore reducing P loads to Sandusky Bay is critically important. However, as best management practices are developed for P mitigation in the Great Lakes basin, managers must be aware of the short-term implications of not managing N loading as our study clearly indicates that for much of the growing season, N inputs may significantly impact bloom size and toxicity. We used metagenomic techniques to take a snapshot of the microbial population potentially involved in N fixation in Sandusky Bay. Furthermore, more research needs to be conducted to better understand the ecological relationships that allow a nondiazotrophic cyanobacterium like *Planktothrix* to dominate in a system that is severely N limited for much of the growing season.

■ ASSOCIATED CONTENT

Supporting Information

Information on initial chlorophyll *a* concentrations and microcystins at EC 1163 and EC 1198 as well as detailed statistical information on each experiment conducted for both

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

sites is located in the Supplemental files. Further, the phylogenetic tree built using the *ntcA* gene is also available. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00799.

AUTHOR INFORMATION

Corresponding Author

*Phone: (734) 741-2286; fax: (734) 741-2055; e-mail: timothy.davis@noaa.gov.

Present Address

[§]NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan 48108, United States.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the captain and crew of the CCGS *Limnos* and Environment Canada's Technical Operations personnel for field assistance, and Clemons Boats (Sandusky, OH) for providing access to field sampling. We thank Dr. Rick Bourbonniere, Mr. Jack Calder, Mr. Tarn Preet Parmar, Mr. Craig Irwin, Mr. Purnank Shah, Mrs. Shelby Grassick, and Mr. Seth Schulte for field and laboratory assistance. We thank the three anonymous reviewers for valuable feedback on our original submission. This manuscript is NOAA-GLERL contribution number 1765. This article includes research supported by funds from Environment Canada's Great Lakes Nutrient Initiative awarded to SW and awards by Ohio Sea Grant (R/ER-104) and the Ohio Water Resources Center G12AP20099 (subaward 60039769) to GSB. The work conducted by the U.S. Department of Energy Joint Genome Institute was supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02−05CH11231 and Community Sequencing Project 723 (RMM and GSB).

REFERENCES

(1) Fuller, K.; Shear, H.; Wittig, J. *The Great Lakes: An Environmental Atlas and Resource Book*; Government of Canada United States Environmental Protection Agency: Toronto, ON, 2002.

(2) Munawar, M.; Munawar, I. F.; Dermott, R.; Niblock, H.; Carou, S. Is Lake Erie a resilient ecosystem? . *Aquat. Ecosyst. Health Manage.* **2002**, *5*, 79−93.

(3) *A Balanced Diet for Lake Erie: Reducing Phosphorus Loadings and Harmful Algal Blooms*, Report of the Lake Erie Ecosystem Priority; International Joint Commission, 2014.

(4) Rosa, F.; Burns, N. M. Lake Erie central basin oxygen depletion changes from 1929−1980. *J. Great Lakes Res.* **1987**, *13*, 684−696.

(5) Makarewicz, J. C.; Bertram, P. Evidence for the restoration of the Lake Erie ecosystem. *Bioscience* **1991**, *41*, 216−223.

(6) Bertram, P. E. Total phosphorus and dissolved oxygen trends in the central basin of Lake Erie, 1970−1991. *J. Great Lakes Res.* **1993**, *19*, 224−236.

(7) DePinto, J. V.; Young, T. C.; McIlroy, L. M. Impact of phosphorus control measures on water quality of the Great Lakes Environ. *Sci. Technol.* **1986**, *20*, 752−759.

(8) Makarewicz, J. C.; Lewis, T.; Bertram, P. *Phytoplankton and zooplankton composition, abundance and distribution and trophic interactions: Offshore region of lakes Eri, eLake Huron, and Lake Michigan*, 1985 CIESIN; U.S. Environmental Protection Agency, 1989; p 15.

(9) Stumpf, R. P.; Wynne, T. T.; Baker, D. B.; Fahnenstiel, G. L. Interannual variability of cyanobacterial blooms in Lake Erie. *PLoS One* **2012**, *7*, e42444.

(10) Michalak, A. M.; Anderson, E. J.; Beletsky, D.; Boland, S.; Bosch, N. S.; Bridgeman, T. B.; et al. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 6448−6452.

(11) Steffen, M. M.; Belisle, B. S.; Watson, S. B.; Boyer, G. L.; Wilhelm, S. W. Status, causes and controls of cyanobacterial blooms in Lake Erie. *J. Great Lakes Res.* **2014**, *40*, 215−225.

(12) Ho, J. C.; Michalek, A. M. Challenges in tracking harmful algal blooms: A synthesis of evidence from Lake Erie. *J. Great Lakes Res.* **2015**, DOI: 10.1016/j.jglr.2015.01.001.

(13) Schindler, D. W. Evolution of phosphorus limitation in lakes. *Science* **1977**, *195*, 260−262.

(14) Schindler, D. W.; Hecky, R. E.; Findlay, D. L.; Stainton, M. P.; Parker, B. R.; Paterson, M.; Beaty, K. G.; Lyng, M.; Kasian, S. E. M. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37 year whole ecosystem experiment. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11254−11258.

(15) Scavia, D.; Allan, J. D.; Arend, K. K.; Bartell, S.; Beletsky, D.; Bosch, N. S.; Brandt, S. B.; Briland, R. D.; Daloğlu, I.; DePinto, J. V.; et al. Assessing and addressing the re-eutrophication of Lake Erie: Central basin hypoxia. *J. Great Lakes Res.* **2014**, *40*, 226−246.

(16) Davis, T. W.; Harke, M. J.; Marcoval, M. A.; Goleski, J.; Orano-Dawson, C.; Berry, D. L.; Gobler, C. J. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. *Aquat. Microb. Ecol.* **2010**, *61*, 149−162.

(17) Paerl, H. W. Mitigating harmful cyanobacterial blooms in a human-and climatically-impacted world. *Life* **2014**, *4*, 988−1012.

(18) Müller, S.; Mitrovic, M. M. Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. *Hydrobiology* **2015**, *744*, 255−269.

(19) Nicholls, K. H.; Hopkins, G. J. Recent changes in Lake Erie (north shore) phytoplankton: Cumulative impacts of phosphorus loading reductions and zebra mussel introduction. *J. Great Lakes Res.* **1993**, *19*, 637−647.

(20) Conroy, J. D.; Kane, D. D.; Dolan, D. M.; Edwards, W. J.; Charlton, M. N.; Culver, D. A. Temporal trends in Lake Erie plankton biomass: Roles of external phosphorus loading and dreissenid mussels. *J. Great Lakes Res.* **2005**, *31*, 89−110.

(21) Chaffin, J. D.; Bridgeman, T. B.; Bade, D. L. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. *Adv. Microbiol.* **2013**, *3*, 16−26.

(22) Porta, D.; Fitzpatrick, M. A. J.; Haffner, G. D. Annual variability of phytoplankton primary production in the western basin of Lake Erie (2002−2003). *J. Great Lakes Res.* **2005**, *31*, 63−71.

(23) Davis, T. W.; Koch, F.; Marcoval, M. A.; Wilhelm, S. W.; Gobler, C. J. Mesozooplankton and microzooplankton grazing during cyanobacterial blooms in the western basin of Lake Erie. *Harmful Algae* **2012**, *15*, 26−35.

(24) Vanderploeg, H. A.; Liebig, J. R.; Carmichael, W. W.; Agy, M. A.; Johengen, T. H.; et al. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 1208−1221.

(25) Conroy, J. D.; Culver, D. A. Do dreissenid mussels affect Lake Erie ecosystem stability process? *Am. Midl. Nat.* **2005**, *153*, 20−32.

(26) Harke, M. J.; Gobler, C. G. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. *PLoS One* **2013**, *8*, e69834 DOI: 10.1371/journal.pone.0069834.

(27) Davis, T. W.; Watson, S. B.; Rozmarynowycz, M. J.; Ciborowski, J. J. H.; McKay, R. M.; Bullerjahn, G. S. Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. *PLoS One* **2014**, *9*, e106093.

(28) Kurmayer, R.; Blom, J. F.; Deng, L.; Pernthaler, J. Integrating phylogeny, geographic niche partitioning and secondary metabolite synthesis in bloom-forming *Planktothrix*. *ISME J.* **2014**, 1−13.

(29) Conroy, J. D.; Quinlan, E. L.; Kane, D. D.; Culver, D. A. *Cylindrospermopsis* in Lake Erie: testing its association with other cyanbacterial genera and major limnological parameters. *J. Great Lakes Res.* **2007**, *33*, 519−535.
(30) Kutovaya, O. A.; McKay, R. M. L.; Beall, B. F. N.; Wilhelm, S. W.; Kane, D. D.; Chaffin, J. D.; Bridgeman, T. B.; Bullerjahn, G. S. Evidence against fluvial seeding of toxic blooms of *Microcystis* spp. in Lake Erie's western basin. *Harmful Algae* **2012**, *15*, 71−77.
(31) Wacklin, P.; Hoffmann, L. Komárek. Nomenclatural validation of the genetically revised cyanobacterial genus *Dolichospermum* (Ralfs ex Bornet et Flahault). *comb. nova. Fottea* **2009**, *9*, 59−64.
(32) Watson, S. B.; Ridal, J.; Boyer, G. L. Taste and odour and cyanobacterial toxins: Impairment, prediction, and management in the Great Lakes. *Can. J. Fish. Aquat. Sci.* **2008**, *65*, 1779−1796.
(33) Rinta-Kanto, J. M.; Wilhelm, S. W. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. *Appl. Eviron. Micorbiol.* **2006**, *72*, 5083−5085.
(34) Chaffin, J. D.; Bridgeman, T. B. Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. *J. Appl. Phycol* **2013**, DOI: 10.1007/s10811-013-0118-0.
(35) U.S. EPA. *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.
(36) Fischer, W. J.; Garthwaite, I.; Miles, C. O.; Ross, K. M.; Aggen, J. B.; Chamberlin, A. R.; Towers, N. R.; Dietrich, D. R. Congener-independent immunoassay for microcystins and nodularins. *Environ. Sci. Technol.* **2001**, *35*, 4849−4856.
(37) Lund, J. W. G.; Kippling, C.; le Cren, E. D. The inverted microscope method of estimating algal numbers and the statistical basis for the estimation by counting. *Hydrobiol.* **1958**, *11*, 144−170.
(38) Rott, E. Some results from phytoplankton counting intercalibrations. *Schweiz Z. Hydrol.* **1981**, *24*, 15−24.
(39) Hamilton, P. The revised edition of a computerized counter for plankton, periphyton and sediment diatom analysis. *Hydrobiol.* **1990**, *194*, 23−30.
(40) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403−410.
(41) Kumar, S.; Nei, M.; Dudley, J.; Tamura, K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings Bioinf.* **2007**, *9*, 299−306.
(42) Penno, S.; Lindell, D.; Post, A. F. Diversity of *Synechococcus* and *Prochlorococcus* populations determined from DNA sequences of the N-regulatory gene *ntcA*. *Environ. Microbiol.* **2006**, *8*, 1200−1211.
(43) Rinta-Kanto, J. M.; Konopko, E. A.; DeBruyn, J. M.; Bourbonniere, R. A.; Boyer, G. L.; Wilhelm, S. W. Lake Erie *Microcystis*: Relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful Algae* **2009a**, *8*, 665−673.
(44) Gobler, C. J.; Davis, T. W.; Coyne, K. J.; Boyer, G. L. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. *Harmful Algae* **2007**, *6*, 119−133.
(45) Kurmayer, R.; Gumpenberger, M. Diversity of microcystin genotypes among populations of the filamentous cyanobacteria *Planktothrix rubescens* and *Planktothrix agardhii*. *Mol. Ecol.* **2006**, *15*, 3849−3861.
(46) Briand, E.; Gugger, M.; Francois, J.-C.; Bernard, C.; Humbert, J.-F.; Quiblier, C. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (Cyanobacterium) population. *Appl. Environ. Microbiol.* **2008**, *74*, 3839−3848.
(47) Sabart, M.; Pobel, D.; Briand, E.; Combourieu, B.; Salencon, M. J.; Humbert, J. F.; Latour, D. Spatiotemporal variations in microcystin concentrations and in the proportions of microcystin-producing cells in several *Microcystis aeruginosa* populations. *Appl. Environ. Microbiol.* **2010**, *76*, 4750−4759.
(48) Oberhaus, L.; Briand, J.-F.; Leboulanger, C.; Jacquet, S.; Humbert, J.-F. Comparative effects of the quality and quantity of light and of temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. *J. Phycol.* **2007**, *43*, 1191−1199.
(49) Donald, D. B.; Bogard, M. J.; Finlay, K.; Leavitt, P. R. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnol. Oceanogr.* **2011**, *56*, 2161−2175.
(50) Donald, D. B.; Bogard, M. J.; Finlay, K.; Bunting, L.; Leavitt, P. R. Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. *PLoS One* **2013**, *8*, e53277 DOI: 10.1371/journal.pone.0053277.
(51) Berger, C. Occurrence of *Oscillatoriacea agardhii* Gom. In some shallow, eutrophic lakes. *Verh. - Int. Ver. Theor. Angew. Limnol.* **1975**, *19*, 2689−2697.
(52) Scheffer, M.; Rinaldi, S.; Gragnani, A.; Mur, L. R.; van Nes, E. H. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology* **1997**, *78*, 272−282.
(53) Paerl, H. W.; Xu, H.; Hall, N. S.; Zhu, G.; Qin, B.; et al. Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: will nitrogen reductions cause replacement of non-N_2 fixing by N_2 fixing taxa? *PLoS One* **2014**, *9*, e113123 DOI: 10.1371/journal.pone.0113123.
(54) Smith, V. H. Low nitrogen to phosphorus rations favour dominance by blue-green algae in lake phytoplankton. *Science* **1983**, *221*, 669−671.
(55) Jensen, J. P.; Jeppesen, E.; Olrik, K.; Kristensen, P. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Can. J. Fish. Aquatic. Sci.* **1994**, *51*, 1692−1699.
(56) Downing, J. A.; Watson, S. B.; McCauley, E. Predicting cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 1905−1908 DOI: 10.1139/f01-143.
(57) O'Neil, J. M.; Davis, T. W.; Burford, M. A.; Gobler, C. J. The rise of harmful cyanobacteria blooms (CHABs): Role of eutrophication and climate change in freshwater, estuarine and marine ecosystems. *Harmful Algae* **2012**, *14*, 313−334.
(58) Gao, X.; Heath, R. T. Relationship between labile dissolved organic carbon (LDOC), bacterioplankton cell phosphorus quota, and bacterial phosphate uptake rate in lakes. *J. Great Lakes Res.* **2005**, *31* (suppl. 2), 125−137.
(59) Paerl, H. W.; Millie, D. F. Physiological ecology of toxic cyanobacteria. *Phycologia* **1996**, *35*, 160−167.
(60) Steppe, T. F.; Olson, J. B.; Paerl, H. W.; Litaker, R. W.; Belnap, J. Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiol. Ecol.* **1996**, *21*, 149−156.
(61) Bell, W. H.; Mitchell, R. Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bull.* **1972**, *143*, 265−277.
(62) Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Yoshida, F.; Ueno, Y.; Watanabe, M. F. Enzyme immunoassay for the direct determination of microcystins in environmental water. *J. AOAC Int.* **1997**, *80*, 408−417.
(63) Tillett, D.; Dittmann, E.; Erhard, M.; von Döhren, H.; Börner, T.; Neilan, B. A. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: An integrated peptide−polyketide synthetase system. *Chem. Biol.* **2000**, *7*, 753−764.
(64) Van de Waal, D. B.; Verspagen, J. M. H.; Lurling, M.; van Donk, E.; Visser, P. M.; et al. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. *Ecol. Lett.* **2009**, *12*, 1326−1335.
(65) Van de Waal, D. B.; Smith, V. H.; Declerck, S. A. J.; Stam, E. C. M.; Elser, J. J. Stoichiometric regulation of phytoplankton toxins. *Ecol. Lett.* **2014**, *17*, 736−742.
(66) Orr, P. T.; Jones, G. J. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* **1998**, *43*, 1604−1614.
(67) Millennium Ecosystem Assessment. *Ecosystems and Human Well-Being, Vol. 1. Current State and Trends: Findings of the Condition and Trends Working Group (Millennium Assessment Series)*; Island Press: Washiington, DC, 2005.

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207

(68) Leavitt, P. R.; Brock, C. S.; Ebel, C.; Patoine, A. Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in central North America. *Limnol. Oceanogr.* **2006**, *51*, 2262−2277.
(69) Bunting, L.; Leavitt, P. R.; Gibson, C. E.; McGee, E. J.; Hall, V. A. Degradation of water quality in Lough Neagh, Northern Ireland, by diffuse nitrogen flux from a phosphorus-rich catchment. *Limnol. Oceanog.* **2007**, *52*, 354−369.
(70) Turpin, D. H.; Layzell, D. B.; Elrifi, I. R. Modeling the C-economy of *Anabaena flos-aquae* - Estimates of establishment, maintenance, and active costs associated with growth on NH_3, NO_3^-, and N_2. *Plant Physiology* **1985**, *78*, 746−752.
(71) Barica, J.; Kling, H.; Gibson, J. Experimental manipulation of algal bloom composition by nitrogen addition. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 1175−1183.
(72) Levine, S. N.; Schindler, D. W. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Can. J. Fish. Aquat. Sci.* **1999**, *56*, 451−466.
(73) Finlay, K.; Patoine, A.; Donald, D. B.; Bogard, M. J.; Leavitt, P. R. Experimental evidence that pollution with urea can degrade water quality in phosphorus-rich lakes of the Northern Great Plains. *Limnol. Oceanogr.* **2010**, *55*, 1213−1230.
(74) Lathrop, R. C. Evaluation of whole-lake nitrogen-fertilization for controlling blue-green-algal blooms in a hypereutrophic lake. *Can. J. Fish. Aquat. Sci.* **1988**, *45*, 2061−2075.
(75) Monchamp, M.-E.; Pick, F. R.; Beisner, B. E.; Maranger, R. Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. *PLoS One* **2014**, *9*, e85573 DOI: 10.1371/journal.pone.0085573.

DOI: 10.1021/acs.est.5b00799
Environ. Sci. Technol. 2015, 49, 7197−7207